

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT:
 - (A) Nico Cerletti
- (ii) TITLE OF INVENTION: New process for the production of biologically active protein
- (iii) NUMBER OF SEQUENCES: 13
- (iv) CORRESPONDENCE ADDRESS
 - (A) ADRESSEE: Novartis Patent Department
 - (B) STREET: 564 Morris Avenue
 - (C) CITY: Summit
 - (D) STATE: New Jersey
 - (E) COUNTRY: USA
 - (F) ZIP: 07901
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: 08/776,444
 - (B) FILING DATE: 24-JAN-97
 - (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: PCT/EP95/02719
 - (B) FILING DATE: 12-Jul-95
 - (A) APPLICATION NUMBER: EPO 94810439.3
 - (B) FILING DATE: 25-Jul-94
 - (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Pfeiffer, Hesna J. .
 - (B) REGISTRATION NUMBER: 22640
 - (C) REFERENCE/DOCKET NUMBER: 4-20039C/C1C1/USN
 - (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (908) 522-6940
 - (B) TELFAX: (908) 522-6955
- (2) INFORMATION FOR SEQ ID NO: 1:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 339 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: cDNA to mRNA
 - (iii) HYPOTHETICAL: NO

	(ix	(1	A) N B) L	E: AME/1 OCAT: THER	ION:	13		:/pr	oduct	t= "]	human	n TG:	F-bei	tal"		
	(xi) SE	QUEN	CE DI	ESCR	IPTI	ON:	SEQ :	ID N	0: 1	:					
				AAC Asn 5												48
				TAC Tyr												96
				AAG Lys												144
	-			AGC Ser							-	-				192
				AAC Asn												240
				CCG Pro 85												288
				CTG Leu												336
TGA																339
(2)		(i) S (<i>I</i> (E	SEQUI A) LI 3) T	FOR ENCE ENGTI YPE: OPOLO	CHAI I: 1: ami:	RACTI 12 ar	ERIST mino cid	rics								
				LE TY		_		SEQ :	ID NO	D: 2	ŧ					
Ala 1	Leu	Asp	Thr	Asn 5	Tyr	Cys	Phe	Ser	Ser 10	Thr	Glu	Lys	Asn	Cys 15	Cys	
Val	Arg	Gln	Leu	Tyr	Ile	Asp	Phe	Arg	Lys	Asp	Leu	Gly	Trp	Lys	Trp	

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(B) CLONE: E. coli LC137/pPLMu.hTGF-beta1 (DSM 5656)

(vii) IMMEDIATE SOURCE:

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	His	Glu 35	Pro	Lys	Gly	Tyr	His 40	Ala	Asn	Phe	Cys	Leu 45	Gly	Pro	Cys	
Pro	Tyr 50	Ile	Trp	Ser	Leu	Asp 55	Thr	Gln	Tyr	Ser	Lys 60	Val	Leu	Ala	Leu	
Tyr 65	Asn	Gln	His	Asn	Pro 70	Gly	Ala	Ser	Ala	Ala 75	Pro	Cys	Cys	Val	Pro 80	
Gln	Ala	Leu	Glu	Pro 85	Leu	Pro	Ile	Val	Tyr 90	Tyr	Val	Gly	Arg	Lys 95	Pro	
Lys	Val	Glu	Gln 100	Leu	Ser	Asn	Met	Ile 105	Val	Arg	Ser	Cys	Lys 110	Cys	Ser	
(2)	INFO	ORMA:	rion	FOR	SEQ	ID N	10 : 3	3:								
	(i)	(<i>I</i> (I	A) LE 3) TY C) ST	engti (PE : [RANI	HARAC H: 33 nucl DEDNE DGY:	89 ba leic ESS:	ase p acid doub	pairs 1	3							
	(ii)	MOI	LECUI	E TY	PE:	CDNA	A to	mRNA	Ā							
(vii)				OURC		L LC:	Լ37/բ	PLMu	ı.hT0	F-be	eta2	(DSN	15657	7)	,
	(ix)		ATURI	E :												
		(I	3) LO	CAT	CEY: ON:1	33		:/pro	oduct	:= "}	numar	ı TGI	₹-bet	:a2"		
	(xi)	(I (I	3) LO O) OT	CATI THER	ON:1	L33 DRMAT	NOI					n TGI	₹-bet	:a2"		
GCT Ala	TTG	(I (I SE(GAT	B) LO D) OT QUENC	CE DE	ON:1	DRMAT	ON: S	SEQ 1	D NO): 3: GTG	CAG	GAT	AAT	TGC		48
Ala	TTG Leu CGT	GAT Asp 115	O) OT	CE DE GCC Ala	ON: 1 INFO ESCRI TAT Tyr ATT	DRMAT PTIC TGC Cys	TION: STITE Phe 120	SEQ I	AAT Asn AGG	GTG Val	CAG Gln CTA	GAT Asp 125 GGG	AAT Asn TGG	TGC Cys AAA	Cys TGG	48 96
Ala CTA Leu ATA	TTG Leu CGT Arg 130 CAC	GAT Asp 115 CCA Pro	GCG Ala	CE DE GCC Ala TAC Tyr	ON:1 INFO	TGC Cys GAT Asp 135	TION: STITT Phe 120 TTC Phe	AGA Arg AAG Lys	AAT Asn AGG Arg	GTG Val GAT Asp	CAG Gln CTA Leu 140	GAT Asp 125 GGG Gly	AAT Asn TGG Trp	TGC Cys AAA Lys GCA	TGG Trp	
CTA Leu ATA Ile 145 CCG	TTG Leu CGT Arg 130 CAC His	GAT Asp 115 CCA Pro GAA Glu	QUENC GCG Ala CTT Leu CCC Pro	CE DE GCC Ala TAC Tyr AAA Lys	CON: 1 INFO	TGC Cys GAT Asp 135 TAC Tyr	TTT Phe 120 TTC Phe AAT ASN	AGA Arg AAG Lys GCC Ala	AAT ASN AGG Arg AAC ASN	GTG Val GAT Asp TTC Phe 155	CAG Gln CTA Leu 140 TGT Cys	GAT Asp 125 GGG Gly GCT Ala	AAT Asn TGG Trp GGA Gly	TGC Cys AAA Lys GCA Ala	TGG Trp TGC Cys 160	96

	GAT Asp														
	ATT Ile 210														
TAA															
(2)	INFO	ORMA'	rion	FOR	SEQ	ID 1	NO: 4	1 :							
		(<i>I</i>	SEQUI A) Li B) T'	ENGTI YPE :	i: 13 amir	L2 ar	nino cid								
			LECUI QUEN(-		SEQ I	ID NO): 4:					
Ala 1	Leu	Asp	Ala	Ala 5	Tyr	Cys	Phe	Arg	Asn 10	Val	Gln	Asp	Asn	Cys 15	Cys
Leu	Arg	Pro	Leu 20	Tyr	Ile	Asp	Phe	Lys 25	Arg	Asp	Leu	Gly	Trp 30	Lys	Trp
Ile	His	Glu 35	Pro	Lys	Gly	Tyr	Asn 40	Ala	Asn	Phe	Cys	Ala 45	Gly	Ala	Cys
Pro	Tyr 50	Leu	Trp	Ser	Ser	Asp 55	Thr	Gln	His	Ser	Arg 60	Val	Leu	Ser	Leu
Tyr 65	Asn	Thr	Ile	Asn	Pro 70	Glu	Ala	Ser	Ala	Ser 75	Pro	Сув	Cys	Val	Ser 80
Gln	Asp	Leu	Glu	Pro 85	Leu	Thr	Ile	Leu	Tyr 90	Tyr	Ile	Gly	Lys	Thr 95	Pro
Lys	Ile	Glu	Gln 100	Leu	Ser	Asn	Met	Ile 105	Val	Lys	Ser	Суз	Lys 110	Cys	Ser
(2)	INFO	ORMA:	rion	FOR	SEQ	ID 1	10: 5	ō:							
	(i)	(<i>I</i>	QUENCA) LI B) T' C) S' D) TC	ENGTI PE : PRANI	H: 33 nucl	89 ba leic ESS:	ase p acio douk	pairs 1	5						•
	(ii)	MOI	LECUI	LE TY	PE:	cDN	A to	mRNA	A						
	(vii)		MEDIZ				i i.c	127/*	n DT .M·	. ከምር	iF_b	at a ?	/Dev	1 565	: o \

(B) CLONE: E. coli LC137/pPLMu.hTGF-beta3 (DSM 5658)

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336

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(ix) FEATURE: (A) NAME/KEY: CDS

		(1	0) OI	THER	INFO	ORMAT	CION	:/pro	oduct	:= "}	numar	n TGI	F-bet	:a3"		
	(xi)	SEÇ	QUENC	CE DI	ESCRI	[PTIC	ON: S	SEQ :	ID NO	D: 5	:					
				AAT Asn												48
				TAC Tyr												96
-				AAG Lys												144
				AGT Ser 165												192
				AAC Asn												240
				CCC Pro												288
	-			CTC Leu												336
TGA																339
(2)	INFO	ORMAT	rion	FOR	SEQ	ID 1	10: 6	5:								

- - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 112 amino acids
 - (B) TYPE: amino acid

(B) LOCATION: 1..336

- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Ala Leu Asp Thr Asn Tyr Cys Phe Arg Asn Leu Glu Glu Asn Cys Cys 5 10

Val Arg Pro Leu Tyr Ile Asp Phe Arg Gln Asp Leu Gly Trp Lys Trp

Val His Glu Pro Lys Gly Tyr Tyr Ala Asn Phe Cys Ser Gly Pro Cys

Pro Tyr Leu Arg Ser Ala Asp Thr Thr His Ser Thr Val Leu Gly Leu 55 50

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	• • • • • • • • • • • • • • • • • • •
Tyr Asn 1	Thr Leu Asn Pro Glu Ala Ser Ala Ser Pro Cys Cys Val Pro 70 75 80
Gln Asp	Leu Glu Pro Leu Thr Ile Leu Tyr Tyr Val Gly Arg Thr Pro 85 90 95
Lys Val (Glu Gln Leu Ser Asn Met Val Val Lys Ser Cys Lys Cys Ser 100 105 110
(2) INFO	RMATION FOR SEQ ID NO: 7:
(i)	SEQUENCE CHARACTERISTICS: (A) LENGTH: 336 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: double (D) TOPOLOGY: linear
(ii)	MOLECULE TYPE: other nucleic acid
TGF-bet	(A) DESCRIPTION: /desc = "recombinant hybrid DNA of cal and TGF-beta3 DNA"
(vii)	IMMEDIATE SOURCE: (B) CLONE: E. coli LC137/pPLMu.TGF-beta1(44/45)beta3
(ix)	<pre>FEATURE: (A) NAME/KEY: mat_peptide (B) LOCATION:1132 (D) OTHER INFORMATION:/product= "N-terminal 44 amino</pre>
(ix)	FEATURE: (A) NAME/KEY: mat_peptide (B) LOCATION:133336 (D) OTHER INFORMATION:/product= "C-terminal 68 amino acids of human TGF-beta3"
(ix)	FEATURE: (A) NAME/KEY: CDS (B) LOCATION:1336 (D) OTHER INFORMATION:/product= "hybrid TGF-beta named TGF-beta1-3"
(xi)	SEQUENCE DESCRIPTION: SEQ ID NO: 7:
	GAC ACC AAC TAT TGC TTC AGC TCC ACG GAG AAG AAC TGC TGC Asp Thr Asn Tyr Cys Phe Ser Ser Thr Glu Lys Asn Cys Cys 5 10 15
	CAG CTG TAC ATT GAC TTC CGC AAG GAC CTC GGC TGG AAG TGG 96 Gln Leu Tyr Ile Asp Phe Arg Lys Asp Leu Gly Trp Lys Trp 20 25 30
	GAG CCC AAG GGC TAC CAT GCC AAC TTC TGC TCA GGC CCT TGC Glu Pro Lys Gly Tyr His Ala Asn Phe Cys Ser Gly Pro Cys 40 45

•																•	
						GAC Asp 55											192
						GAA Glu											240
					-	ACC Thr											288
				-		AAC Asn											336
(2)					_	ID 1											
		(<i>I</i>	A) LI 3) T	ENGTI	H: 13 amin	RACTI 12 am 10 am 1ine	nino cid										
						prot PTI		SEQ]	ID NO): 8:	:						
Ala 1	Leu	Asp	Thr	Asn 5	Tyr	Cys	Phe	Ser	Ser 10	Thr	Glu	Lys	Asn	Cys 15	Cys		
Val	Arg	Gln	Leu	Tyr	Ile	Asp	Phe	Arg	Lys	Asp	Leu	Gly	Trp	Lys	Trp		

20 25 30

Ile His Glu Pro Lys Gly Tyr His Ala Asn Phe Cys Ser Gly Pro Cys 35 40 45

Pro Tyr Leu Arg Ser Ala Asp Thr Thr His Ser Thr Val Leu Gly Leu 50 55 60

Tyr Asn Thr Leu Asn Pro Glu Ala Ser Ala Ser Pro Cys Cys Val Pro 65 ' 70 75 80

Gln Asp Leu Glu Pro Leu Thr Ile Leu Tyr Tyr Val Gly Arg Thr Pro 85 90 95

Lys Val Glu Gln Leu Ser Asn Met Val Val Lys Ser Cys Lys Cys Ser 100 105 110

(2) INFORMATION FOR SEQ ID NO: 9:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 336 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "recombinant hybrid DNA

coding for hybrid TGF-beta2-3"

(vii) IMMEDIATE SOURCE:

(B) CLONE: E. coli LC137/pPLMu.TGF-beta2(44/45)beta3

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
- (B) LOCATION:1..132
- (D) OTHER INFORMATION:/product= "N-terminal 44 amino acids of human TGF-beta2"

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
- (B) LOCATION:133..336
- (D) OTHER INFORMATION:/product= "C-terminal 68 amino acids of human TGF-beta3"

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION:1..336
- (D) OTHER INFORMATION:/product= "hybrid TGF-beta2-3"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

 	 		TGC Cys					48
 	 		GAT Asp			_		96
			TAC Tyr					144
			GAC Asp 55					192
			GAA Glu					240
			ACC Thr					288
			AAC Asn					 336

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 112 amino acids

- (B) TYPE: amino acid
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Ala Leu Asp Ala Ala Tyr Cys Phe Arg Asn Val Gln Asp Asn Cys Cys

1 10 15

Leu Arg Pro Leu Tyr Ile Asp Phe Lys Arg Asp Leu Gly Trp Lys Trp
20 25 30

Ile His Glu Pro Lys Gly Tyr Asn Ala Asn Phe Cys Ser Gly Pro Cys
35 40 45

Pro Tyr Leu Arg Ser Ala Asp Thr Thr His Ser Thr Val Leu Gly Leu 50 55 60

Tyr Asn Thr Leu Asn Pro Glu Ala Ser Ala Ser Pro Cys Cys Val Pro 65 70 75 80

Gln Asp Leu Glu Pro Leu Thr Ile Leu Tyr Tyr Val Gly Arg Thr Pro 85 90 95

Lys Val Glu Gln Leu Ser Asn Met Val Val Lys Ser Cys Lys Cys Ser 100 105 110

- (2) INFORMATION FOR SEQ ID NO: 11:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 336 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: other nucleic acid
 - (A) DESCRIPTION: /desc = "recombinant hybrid DNA coding for hybrid TGF-beta3-2"
 - (vii) IMMEDIATE SOURCE:
 - (B) CLONE: E. coli LC137/pPLMu.TGF-beta3(44/45)beta2
 - (ix) FEATURE:
 - (A) NAME/KEY: mat_peptide
 - (B) LOCATION:1..132
 - (D) OTHER INFORMATION:/product= "N-terminal 44 amino acids of human TGF-beta3"
 - (ix) FEATURE:
 - (A) NAME/KEY: mat_peptide
 - (B) LOCATION: 133..336
 - (D) OTHER INFORMATION:/product= "C-terminal 68 amino acids of human TGF-beta2"
 - (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION:1..336



(D) OTHER INFORMATION:/product= "hybrid TGF-beta3-2"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

	-			TGC Cys						 48
				GAC Asp						96
 	_	 _	-	TAC Tyr						 144
				GAC Asp 55						192
				GAA Glu						240
				ACC Thr			_	_		288
				AAT Asn						336

(2) INFORMATION FOR SEQ ID NO: 12:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 112 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Ala Leu Asp Thr Asn Tyr Cys Phe Arg Asn Leu Glu Glu Asn Cys Cys

1 5 10 15

Val Arg Pro Leu Tyr Ile Asp Phe Arg Gln Asp Leu Gly Trp Lys Trp 20 25 30

Val His Glu Pro Lys Gly Tyr Tyr Ala Asn Phe Cys Ala Gly Ala Cys 35 40 45

Pro Tyr Leu Trp Ser Ser Asp Thr Gln His Ser Arg Val Leu Ser Leu 50 60

Tyr Asn Thr Ile Asn Pro Glu Ala Ser Ala Ser Pro Cys Cys Val Ser 65 70 75 80

Gln Asp Leu Glu Pro Leu Thr Ile Leu Tyr Tyr Ile Gly Lys Thr Pro 85 " 90 95

Lys Ile Glu Gln Leu Ser Asn Met Ile Val Lys Ser Cys Lys Cys Ser 100 105 110

(2) INFORMATION FOR SEQ ID NO: 13:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 12 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

CCGGAATTCC GG 12